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Teratogenicity Study of N-Methylpyrrolidone after Dermal Application to Sprague-Dawley Rats

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ABSTRACT

Teratogenicity Study of N-Methylpyrrolidone after Dermal Application to Sprague-Dawley Rats. Becci, P.J., Knickerbocker, M.J., Reagan, E.L., Parent, R.A., and Burnette, L.W. (1982). *Fundam. Appl. Toxicol.* 2:73-76. Teratogenicity studies were performed in rats given N-methylpyrrolidone, a solvent used in chemical processing. Dosages of 75, 237 and 750 mg of N-methylpyrrolidone/kg body weight/day were administered dermally to groups of 25 pregnant Sprague-Dawley rats on days 6 through 15 of gestation. Additionally, the study used a positive dermal control. Hexafluoroacetone, was chosen based on its dermal teratogenic activity. An oral positive control, aspirin, was included in order to add significance to the data generated in the experimental positive dermal control group. All animals were killed and subjected to uterine examination on day 20 of gestation. Maternal toxicity was indicated at 750 mg of N-methylpyrrolidone/kg by reduced body weight gain during gestation. Treatment with N-methylpyrrolidone resulted in dose-dependent brightly colored yellow urine and dry skin. Treatment at the high dosage level resulted in fewer live fetuses per dam, an increase in the percentage of resorption sites and skeletal abnormalities. These effects could be the result of maternal toxicity. There was no evidence of teratogenic effects nor effects on the dams at 75 and 237 mg/kg of body weight.

INTRODUCTION

N-Methylpyrrolidone is a solvent used extensively in chemical processing and is capable of dermal penetration. Schmitt (1976) found that N-methylpyrrolidone caused dose-dependent embryotoxic and teratogenic effects in AJ JENA and C57BL mice when given in single or repeated i.p. doses on various days of gestation. The most pronounced embryotoxic effect of N-methylpyrrolidone was noted after a single i.p. administration of 166 mg/kg was given on the 7th day post-conception. Twenty-three percent of all implanted fetuses died. The same dose level of N-methylpyrrolidone given on the 9th day caused the highest rate of fetal malformations, 18.6%.

In the absence of teratogenicity information by a practical route of exposure for industrial uses, the present study was undertaken to furnish such data by the dermal route. This was judged to be a more significant mode than inhalation, since the material (b.p. 202°C) is of limited volatility.

METHODS

Animals and materials

Young adult Sprague-Dawley rats were obtained from Blue Spruce Farms, Inc., Altamont, New York. Animals were individually housed in wire mesh bottom cages in an environment-controlled room artifically illuminated for 12 hours each day and maintained at 22±2°C. All animals received Charles River RMH Ration and tap water *ad libitum*. N-methylpyrrolidone (lot no. 821) was provided by GAF Corporation, Wayne, New Jersey and was 99.9% pure with a methylamine content of 0.008% and water content of 0.04%. Hexafluoroacetone sesquihydrate (lot no. 021157) was obtained from Aldrich Chemical Company, Inc., Milwaukee, Wisconsin, as a pure (100%) liquid.

Dose range finding study

The dose levels of N-methylpyrrolidone used were 500, 1100 and 2500 mg/kg body weight/day. Hexafluoroacetone, the positive experimental dermal control (Brittelli, et al. 1978), was prepared fresh daily as a 25% solution in water and dosed at a level of 5 mg/kg body weight/day. The amount of material administered to each animal was adjusted during the dosing period according to the most recent body weight. Negative control animals were treated (shaved, weighed, fitted with collars, etc.) the same as all others but received no daily application of the material.

Rats were mated, one male to one female; no male was allowed to impregnate more than one female per group. Insemination was confirmed by observation of a vaginal sperm plug and the day on which the plug was found was designated as day 0 of pregnancy. After mating, five pregnant females were assigned to each treatment group. On day 5 of gestation the application site was prepared by carefully clipping the fur from an area approximately 25 cm^2 on the back of each animal. At that time each female was fitted with a collar designed to prevent ingestion of the test material. The collars were not removed until day 16 of gestation. Beginning on day 6 of

TABLE 1
Dose Range Finding Study:
Dam Body Weight Gain During Gestation

	Treatment (mg/kg BW/day)			
	Control	Hexafluoro-acetone (5)	N-Methyl-pyrrolidone[A] (500)	(1100)
Body Weight Gain (g)[B]	118.7 ±6.6	132.3 ±9.9	127.5 ±4.4	50.0[C] ±12.9

[A]All dams at the high dosage level (2500 mg/kg) died before day 20 of gestation, thus the data is not included.
[B]Group mean ± standard error.
[C]Significantly different from control $p \leq 0.01$.

TABLE 2
Dose Range Finding Study: Summary of Reproduction Data

Treatment (mg/kg BW/day)	No. of Females Pregnant	Viable Offspring (mean/dam)	Implantations (mean/dam)	Resorptions (mean/dam)	Mean Fetus Weight (g)
Control	3	12.3	13.0	0.7	3.1
HFA[A], 5	4	11.5	12.0	0.5	3.4
NMP[A], 500	4	10 8	11.3	0.5	3.2
NMP, 1100	5	0 2[C]	13.2	13.0[C]	2.1[C]
NMP, 2500	4[B]	---	11.3	---	---

[A]HFA, hexafluoroacetone; NMP, N-methylpyrrolidone.
[B]All dams died or aborted before day 20 of gestation.
[C]Significantly different from control $p \leq 0.05$.

TABLE 3
Teratogenicity Study: Dam Body Weight Gain During Gestation

	Treatment (mg/kg BW/day)					
	Water (750)	Hexafluoro-acetone (10)	Asprin (250)	N-Methylpyrrolidone (75)	(237)	(750)
Body Weight Gain (g)[A]	111.3 ±6.5	108.9 ±6.1	94 7[B] ±4.9	123.0 ±3.4	124.3 ±3.3	91.9[B] ±7.8

[A]Groups mean ± standard error.
[B]Significantly different from water treated group (negative control), $p \leq 0.05$.

TABLE 4
Teratogenicity Study: Summary of Reproduction Data

Treatment (mg/kg BW/day)	No. of Females Pregnant	Viable Offspring Mean/dam	Viable Offspring Sex Ratio (M/F)	Implantations (mean/dam)	Resorptions (mean/dam)	Mean Fetus Weight (g)
Water	24	11.2	1.02	11.8	0.6	3.5
HFA[A], 10	24	9.8	1.08	11.3	1.5[B]	3.0[B]
Aspirin, 250	24	7.0[B]	1.19	12.1	5.1[B]	2.1[B]
NMP[A], 75	22	11.9	0.93	12.1	0.3	3.5
NMP, 237	23	12.0	0.94	12.2	0.2	3.5
NMP, 750	24	9.3[B]	0.83	11.0	1.7[B]	2.8[B]

[A]HFA, Hexafluoroacetone; NMP, N-methylpyrrolidone.
[B]Significantly different from water treated group (negative control), $p \leq 0.05$

gestation and continuing daily through day 15 of gestation, the appropriate material was administered dermally to the back of each dam, spread over as much of the application site as possible and rubbed in. The test materials were allowed to remain on the animals for 8 hours per day. At the end of each daily exposure period, the back of each animal was thoroughly washed with water to remove any residual material. No attempt was made to determine the amount of material removed.

All animals were observed daily for general appearance, behavior and mortality. Body weights of dams were taken on days 0, 6, 9, 12, 15 and 20 of gestation.

On day 20 of gestation all females were euthanized with chloroform. The uterine contents of each female were removed and examined. Parameters measured included number of females pregnant, number of fetuses alive or dead, body weight and sex of each fetus; and the number of corpora lutea, implantation sites and resorption sites. The urogenital tract of each female was examined for anatomical normality. All animals that died or were killed when moribund were given a thorough uterine examination.

Teratogenicity study

The dose levels of N-methylpyrrolidone used were 75, 237 and 750 mg/kg body weight/day. Hexafluoroacetone (positive dermal control) was prepared fresh daily as a 25% aqueous solution and administered at a level of 10 mg/kg body weight/day. Aspirin, acetylsalicylic acid, (positive oral control) was prepared fresh weekly as a 2.5% aqueous suspension and administered p.o. at a level of 250 mg/kg body weight/day. Water was dermally applied to negative control animals at a level of 750 mg/kg body weight/day.

All procedures for administration of material, observation of animals, etc., were identical to those described for the *"Dose range finding study"* with the exception of those described below. Subsequent to mating, 25 pregnant females were assigned to each treatment group. After removal and examination of the uterine contents for reproductive performance on day 20 of gestation, an examination of each fetus for teratologic effects was conducted. For soft tissue examinations, one third of the fetuses of each group were fixed in Bouin's solution, sectioned according to the method of Wilson (1965) and examined in detail for soft tissue abnormalities. For skeletal

examinations, the remaining fetuses were fixed in ethyl alcohol, stained with alizarin red (Dawson, 1926) and examined for skeletal defects.

Statistical analysis

Incidences of occurrence were expressed as percent, and comparisons between the negative control and test groups were made using either 95% confidence intervals for proportions or by computations of exact probabilities. Continuous data was analyzed using analysis of variance. All comparisons were made using the dam or litter as the unit of observation rather than the fetus.

RESULTS

Dose range finding study

Treatment with N-methylpyrrolidone at all dosage levels was associated with patches of dry skin at the application site of all animals. The color of the urine in all animals treated with N-methylpyrrolidone was bright yellow. These changes were not observed in control animals. Dam body weight gain during gestation is summarized in Table 1. N-Methylpyrrolidone dosed at a level of 1100 mg/kg or greater caused a significant depression in dam body weight gain.

A summary of the reproduction data is given in Table 2. At the high dosage level of N-methylpyrrolidone, all dams died or spontaneously aborted prior to day 20 of gestation. The middle dosage level (1100 mg/kg) of N-methylpyrrolidone was embryolethal; all but one of 66 fetuses were resorbed. N-Methylpyrrolidone at the low dosage level (500 mg/kg) had no adverse effect on pregnancy, dam body weights, implantations or gestation when compared to the negative controls.

The experimental positive control hexafluoroacetone, at 5 mg/kg had no effect on the reproductive parameters measured when compared to untreated control rats, thus this dosage level was doubled for the teratology study.

Teratology study

The majority of dams treated with N-methylpyrrolidone showed patches of dry skin at the application site, the severity of which increased with dose. Also increasing with dose was the frequency of dams that showed bright yellow colored urine. Only one animal at the low dosage level (75 mg/kg) was noted to have bright yellow colored urine, while all animals at the high dosage level (750 mg/kg) showed the finding. Negative control animals dermally treated with water exhibited neither of these changes.

A summary of dam body weight gain during gestation is given in Table 3. The toxicity of N-methylpyrrolidone at the high dosage level (750 mg/kg) and of aspirin was indicated by a significantly reduced body weight gain in comparison to the negative control animals. Body weight gain of dams at the low and middle dosage levels of N-methylpyrrolidone was comparable to negative controls.

The reproductive parameters of dams is given in Table 4. Treatment with N-methylpyrrolidone at the high dosage level was associated with a statistically significant decrease in the number of viable offspring, an increase in the mean number of resorption sites per dam and a decrease in the mean fetal

TABLE 5
Teratogenicity Study: Summary of Skeletal Data

Findings	Treatment [mg/kg BW/day]					
	Water (750)	Hexafluoro-Acetone (10)	Aspirin (250)	N-Methylpyrrolidone (75)	(237)	(750)
Number of litters (fetuses) examined	24 (187)	23 (159)	18 (109)	22 (108)	23 (192)	22 (156)
Sternabrae						
Incomplete ossification	24 (144)	22 (153)	15 (74)	21 (137)	23 (149)	22 (145)
Bipartite	0	0	1 (1)	1 (1)	0	1 (1)
Fused	0	0	0	1 (1)	0	0
Missing	1 (1)	13 (27)[A]	17 (81)[A]	0	4 (5)	17 (63)[A]
Ribs						
Incomplete ossification	0	1 (1)	1 (1)	0	0	0
Fused/split	0	0	7 (8)[A]	0	0	4 (4)[A]
Wavy	0	10 (18)[A]	2 (4)	1 (1)	0	0
Free floating	0	0	0	0	0	1 (1)
Rudimentary	9 (13)	23 (109)[A]	12 (30)[A]	8 (12)	8 (14)	10 (16)
Extra	0	6 (6)[A]	12 (70)[A]	0	2 (2)	7 (12)[A]
Vertebrae						
Incomplete ossification	12 (16)	23 (122)[A]	18 (10)[A]	11 (18)	14 (26)	18 (38)[A]
Scrambled	0	0	0	0	0	1 (1)
Skull						
Incomplete closing	0	6 (10)[A]	12 (33)[A]	0	0	7 (12)[A]
Fused atlas & exoccipital	0	0	0	0	0	5 (8)[A]
Mandible missing	0	0	0	0	0	1 (1)
Extremities						
Incomplete ossification	1 (1)	18 (59)[A]	12 (49)[A]	0	0	0
Hyoid						
Missing	2 (3)	7 (9)[A]	9 (19)[A]	1 (1)	1 (1)	0
Reduced/incomplete	0	6 (11)[A]	13 (42)[A]	0	1 (1)	3 (3)[A]

[A]Significantly different from water treated group (negative control), $p \leq 0.05$.

TABLE 6
Teratogenicity Study: Summary of Soft Tissue Anomalies

Findings	Treatment [mg/kg BW/day]					
	Water (750)	Hexafluoro-acetone (10)	Aspirin (250)	N-Methylpyrrolidone (75)	(237)	(750)
Number of fetuses examined	24 (82)	23 (76)	19 (60)	22 (81)	23 (83)	21 (67)
Hemorrhage, abdomen	2 (2)	2 (2)	0	6 (9)[A]	4 (4)	2 (2)
Hemorrhage, thorax	0	0	0	0	1(1)	0
Encephalomeningocele	1 (1)	0	19 (30)[A]	0	.0	0
Gastroschisis	0	2 (2)	4 (7)	0	1 (1)	0
Harelip	0	1 (1)	0	0	0	0
Spina bifida	0	0	12 (23)[A]	0	0	0
Exophthalmos	0	0	1 (2)	0	0	0

[A]Significantly different from water treated group (negative control), $p \leq 0.05$.

weight. At the middle and low dosage levels of N-methylpyrrolidone, no effect on pregnancy, implantation or gestation were noted in comparison to negative control rats. Treatment of pregnant dams with either of the two positive controls, hexafluoroacetone or aspirin, caused a significant increase in the mean number of resorptions per dam and a decrease in the mean fetal weight, while only treatment with aspirin caused a significant decrease in the number of viable offspring per dam.

Fetuses from dams receiving the high dosage level of N-methylpyrrolidone exhibited an increased incidence of several skeletal abnormalities (Table 5). Similar abnormalities were observed in the fetuses from dams treated with hexafluoroacetone or aspirin. Soft tissue examinations revealed no dose related differences in the type or frequency of anomalies observed in fetuses from dams treated with N-methylpyrrolidone when compared to negative control fetuses (Table 6). The teratogenic potential of aspirin was demonstrated by an increase in the number of fetuses with soft tissue abnormalities.

DISCUSSION

The acute oral LD_{50} of N-methylpyrrolidone in Sprague-Dawley rats was found to be 4320 mg/kg body weight (unpublished by authors). In the present study the highest dosage level of N-methylpyrrolidone was approximately 1/6 of its acute oral LD_{50} while the middle and low dosage levels were approximately 1/18 and 1/58 of the LD_{50}, respectively. Hexafluroacetone at a dosage level of 10 mg/kg body weight/day appeared to perform satisfactorily as a dermal positive control. The results obtained in our study were comparable to those obtained by Brittelli, et. al. (1979).

The high dose level of N-methylpyrrolidone produced toxic effects in the treated dams, whereas the two lower dosage levels did not result in any such effects. All dams survived the duration of the study. An increased incidence of several skeletal anomalies was observed in the fetuses of dams receiving the high dose (750 mg/kg) of N-methylpyrrolidone. They were missing (unossified) sternebrae, incompletely ossified vertebrae, incomplete skull closure and reduced hyoid. In connection with lowered fetal weights at this level, these findings indicate an overall state of immaturity in fetal development; apparently due to maternal toxicity at this dose. This is supported by fetal weights being significantly lower ($p < 0.05$) in the high dosage level as compared to the water control.

Other skeletal findings at the high dose level such as fused/split ribs, supernumerary ribs and fusion of the atlas and exocipital bones would be considered as malformations. While these changes are indicators of teratogenic potential, it must be noted that these abnormalities occurred only at a level of N-methylpyrrolidone which is maternally toxic. For example, supernumerary ribs have been interpreted by some to be an indicator of teratogenicity (Yasuda and Maeda, 1973). Kimmel and Wilson (1973) noted that maternal stress and embryo-toxicity attributed to maternal treatment at high doses were associated with development of extra ribs.

Karnofsky (1965) first stated the concept now established as Karnofsky's law: almost anything is capable of adversely affecting the conceptus if given at a high enough dose level. Johnson (1981) concluded that a chemical substance would need to be regulated as a developmental hazard only if the embryo is uniquely susceptible to the agent. Agents such as N-methylpyrrolidone that are coeffective teratogens (adversely affecting the embryo but only at a dose level near that adversely affecting the adult) would not necessarily be regulated as a developmental hazard (Johnson, 1981). The regulatory level of this compound should be established on the basis of its toxicity to the adult and with consideration of a modest safety factor to provide protection to the conceptus from this coeffective teratogen.

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